ELEPHANT NECROPSY PROTOCOL

(Elephas maximus and Loxodonta africana)

The American Zoo and Aquarium Association
Elephant Species Survival Plan

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ELEPHANT HERPESVIRUS DISEASE ALERT

The cause of a highly fatal disease of elephants in North American and European Zoos has been identified recently as a new type of herpesvirus. The herpesvirus affects mainly young elephants and usually has a fatal outcome within an hour to a week of onset. Clinical signs are variable and include lethargy, edematous swellings of the head and thoracic limbs, oral ulceration and cyanosis of the tongue. Necropsy findings include extensive cardiac and serosal hemorrhages and edema, hydropericardium, cyanosis of the tongue and oral and intestinal ulcers. Histological features are microhemorrhages with very mild inflammation in the heart, liver and tongue accompanied by intranuclear inclusion bodies in the capillary endothelium. Transmission electron microscopy of the inclusion bodies shows 80-90 nm diameter viral capsids consistent with herpesvirus morphology.

Serological tests have been recently developed (2002) using molecular techniques to express antigens because it has not been possible to cultivate the virus *in vitro*. Some of the epidemiological aspects of the disease are not yet clear and are still under study. Although African elephants are known to carry the virus that is fatal for Asian elephants, there have been a number of cases in Asian elephants in which no direct contact occurred with African elephants. Asian calves (less than two years of age) from different facilities in the U.S. became ill with the clinical signs noted above, and were found to have the herpesvirus by a blood test using polymerase chain reaction (PCR). Of seven elephants that were treated with famciclovir, three recovered. The onset of the disease may be very rapid with few prodromal signs and percute death within 24 to 36 hours. This occurred in 1999-2000 in a six and eight year old Asian elephant that both died even through famciclovir was administered several hours after herpes infection was suspected.

If you suspect an elephant in your care may have died from this disease or shows clinical signs, please contact one of the principals listed below. Consult the Tissue Checklist section of this necropsy protocol for instructions on sending diagnostic samples from any elephants suspected of having this disease. **Serum samples from sick or dead elephants should be obtained for diagnostic testing in any suspected case of herpesvirus infection.**

Contacts: R. J. Montali, Smithsonian National Zoo, Washington DC, W: 202-673-4869,
H: 703-718-2870; Cell: 703-860-0186;Email: montalir@nzp.si.edu
Laura K. Richman, Smithsonian National Zoo, Washington DC, W: 202-673-4869, H: (301) 253-8723; Cell: 410-491-2294; Email: lkrichma@aol.com

ELEPHANT TUBERCULOSIS ALERT

An intense search for lesions of tuberculosis (TB) is encouraged in all elephant necropsies. This should include all elephants that die or are euthanized for other reasons even though TB is not suspected. Be advised that elephant TB is likely to be caused by *Mycobacterium tuberculosis* which is contagious to humans. Therefore be prepared with proper protective apparel, and contain any suspicious organs or lesions as soon as possible.

Ideally, elephants should be bled for serology (ELISA), and trunk wash(es) collected just prior to euthanasia. Elephants that die naturally should have a post mortem trunk wash performed and serum should be harvested from post mortem blood for serological assays. Consult **Guidelines for the Control of Tuberculosis in Elephants 2003** (www.aphis.usda.gov/ac/TBGuidelines2003.html).

All elephants undergoing necropsies should have a careful examination of the tonsillar regions and submandibular lymph nodes for tuberculous appearing lesions. All lymph nodes should be carefully evaluated for lesions since other sites may also be infected (ex. reproductive or gastrointestinal tract). Take any nodes that appear caseous or granulomatous for culture (freeze or ultrafreeze), and fixation (in buffered 10% formalin). In addition, search thoracic organs carefully for early stages of TB as follows: after removal of the lungs and trachea, locate the bronchial nodes at the junction of the bronchi from the trachea. Use clean or sterile instruments to section the nodes. Freeze half of the lymph node and submit for TB culture to NVSL or a laboratory experienced in mycobacterial culture and identification (**even if no lesions are evident**). Submit sections in formalin for histopathology. Carefully palpate the lobes of both lungs from the apices to the caudal borders to detect any firm B-B shot to nodular size lesions. Take sections of any suspicious lesions. Open the trachea and look for nodules or plaques and process as above. Regional thoracic and tracheal lymph nodes should also be examined and processed accordingly. Split the trunk from the tip to its insertion and take samples of any plaques, nodules or suspicious areas for TB diagnosis as above. Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.

For further information on laboratories performing diagnostic tests for TB, consult **Guidelines for the Control of Tuberculosis in Elephants 2003**. In the event of an elephant necropsy (elective or otherwise), please notify Dr. R. J. Montali, AZA-Elephant SSP Pathology Advisor, National Zoo, Washington DC, W: 202-673-4869, H: 703-718-2870; Cell: 703-863-0186; E-mail: montalir@nzp.si.edu, for further instructions and possible participation.

INTERNET SITES

These guidelines and other elephant protocols are available on the internet at the following sites:

1. www.aphis.usda.gov/ac/TBGuidelines2003.html (available to the public)
2. www.aphis.usda.gov/ac/ElephNecropsy2003.html (available to the public)
3. www.aazv.org (available to AAZV members by password)
4. www.elephantcare.org (available to the public)

INTRODUCTION

This protocol is an effort of the Elephant Species Survival Plan (SSP) Propagation Group of the American Zoo and Aquarium Association (AZA). Its purpose is to provide a format for the systematic collection of information and samples that will add to our knowledge of elephants. All North American institutions holding elephants will receive a copy.

We hope that most institutions will not have to face the immense task of performing an elephant necropsy, but should a death occur, it should be viewed as an important learning opportunity. Although it may not be feasible to collect all the information and samples requested, we encourage the collection of as much as possible. With the increased availability of digital cameras, it is strongly recommended that photographs of both normal and pathologic structures be recorded for future reference.

Sample and data collection information is contained in a separate document, **Elephant Research and Tissue Request Protocol.** The *Search List* describes those parts of the anatomy for which data is lacking or about which previous observations need to be confirmed or refuted. The *Measurements Checklist* may seem tedious, but only this type of attention to detail will allow us to expand our knowledge of elephant anatomy. Both of these requested data sets are optional and included in an accompanying document, Elephant Research and Tissue Request Protocol. Some of these observations may be applied to live animals. Therefore, this protocol should be referred to when planning a procedure that might facilitate data collection.

Acquainting oneself with the protocols in both documents (Elephant Necropsy Protocol and Elephant Research and Tissue Request Protocol) and having the necessary equipment ready will facilitate sample collection. It is suggested that a necropsy team be designated in advance; the ability to mobilize skilled individuals quickly will save valuable time particularly in the event of a sudden death. Veterinarians, anatomists, and pathologists from nearby universities may be enlisted to assist the institution's staff. In addition, a list of researchers interested in participating in elephant necropsies is included in this protocol.

A revised Elephant Research and Tissue Request Protocol will be forwarded periodically as new requests are received and projects end. Contact Michele Miller for current requests. A copy of the completed gross pathology protocol with preliminary findings should be sent right after the necropsy and followed by the histopathology and any other lab reports when completed, and digital or color slides to Drs. R.J. Montali and Genny Dumonceaux.

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Home: 703-718-2870; Cell: 703-863-0186
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Disney's Animal Kingdom
Department of Veterinary Services
P.O. Box 10,000
Work: (407) 939-7316 Fax: (407) 938-1909
Email: Michele.Miller@disney.com

Lake Buena Vista, FL 32830-1000

EQUIPMENT CHECKLIST

1. Standard large animal necropsy instruments. Multiple scalpel handles, duplicates or triplicates of other instruments. Extra box of scalpel blades, knife sharpener, and a continual supply of sharp knives.
2. Retractors of various sizes and shapes. Self-retaining retractors with one or two movable arms mounted on a slide bar are most useful if available. .
3. Sterile instruments for culture collection.
4. 10% neutral buffered formalin.
5. 4% buffered glutaraldehyde).
6. Containers for sample collection.
7. Culture swabs, sterile urine cups, glass slides.
8. Serum tubes for blood and urine collection.
9. Aluminum foil and plastic bags for freezing tissues.
10. Labels and waterproof marking pens.
11. Scale for obtaining organ weights.
12. Tape measure (metric), at least 2 meters long.
13. Chain saw, axe, or reciprocating saw to cut through the cranium. Hammers, chisels and handsaws.
14. Hoist/crane.
15. Carts on rollers to move heavy parts.
16. Coveralls, boots, gloves, caps, masks, protective eye and head gear.
17. Accessible water supply with hose.
18. Camera (conventional and/or digital) and film, extra batteries.
19. First aid kit.
20. Surgical masks approved for TB exposure (example: 3M model N95).

LOGISTICS AND NECROPSY TIPS

Heavy equipment may be necessary to move a dead elephant. For an on site necropsy, chains and a tow truck may be sufficient to reposition the animal or to move it a short distance. If the animal must be transported to a remote site, a truck with a hoist will be needed. It may be easier to manipulate the animal onto a flatbed trailer. Vehicles must be able to handle these approximate weights: female Asian: 2,300 - 3,700 kg; male Asian: 3,700 - 4,500 kg; female African: 2,300 - 4,000 kg; male African: 4,100 - 5,000 kg. Trucks can generally be rented or may be available from a telephone company. If a flatbed carrier is used, the animal will need to be strapped to the bed and covered with a tarp (a baseball diamond infield tarp works well). If transportation will be delayed, the carcass can be covered with ice.

If death is imminent or euthanasia is planned, completion of the measurement checklist antemortem will save time at necropsy. Otherwise, measurements should be done as soon after death as possible.

Assigning specific tasks to team members will help the necropsy to proceed in an orderly manner. For example, a team may be assigned to each of these areas: head, forelegs, hindlegs, abdominal region. One person should oversee the collection, labeling, and processing of research materials and any communication concerning research requests. It may be helpful to designate a media spokesperson.

Dissection of the head is best completed after separating it from the body. A good portion of the cranium must be damaged to remove the brain intact; a chain saw, large axe, and chisels are needed to penetrate the thick

cranium. A battery operated reciprocating saw with a replaceable metal cutting blade may be safer and easier to handle. A posterior approach to brain removal can be made by 3 connecting deep cuts with a chain saw in the margins of the flattened triangle formed at the base of the elephant skull. Then remove the bony plate in chunks with a curved crow-bar. Use of a chain saw on bone can be hazardous and cause shrapnel-like fragments to be launched. Protective eye, head and face gear should be worn by the chain saw operator and personnel in the immediate area.

In case an elephant may be unknowingly tuberculous, dissection of the thoracic cavity should always be performed last, and preferably by two people with face masks (hepa-filter preferred) and other protection against Mycobacteria. All other personnel should be dismissed from the area before the thoracic cavity is entered. After the initial incision at the ventral midline is made, one person holds the retractor and the other cuts the tensed skin. Once the sternum is exposed, the ribs are separated at the cartilaginous attachment and adjustable retractors are applied to hold the cavity open. Alternatively, after the abdominal viscera are removed, the diaphragm can be cut from its costosternal attachments and the lungs palpated from a caudal approach for tuberculous nodules, as the lobes are being separated from the closely adhered visceral and parietal pleura. The heart, lungs, and associated structures may then be removed “en bloc”.

ELEPHANT NECROPSY PROTOCOL GROSS EXAMINATION WORKSHEET

Institution/Owner_____

Address_____

Species_____ISIS#_____Studbook#_____
Name_____

Birth date/Age_____Sex_____Weight (Kg)_____
Actual ? Estimate ?

Death date_____Death location_____

Necropsy date_____Necropsy location_____
Post mortem interval_____

Captive Born ? Wild Caught ?

History (clinical signs, circumstances of death, clinical lab work, diet & housing)

_____
_____
_____
_____
_____

GROSS EXAMINATION

(If no abnormalities are noted, mark as normal or not examined (NE); use additional sheets if needed)

General Exam (physical and nutritional condition, skin, body orifices, superficial lymph nodes)

_____
_____
_____
_____
_____
_____

Musculoskeletal System (bones, marrow, joints, muscles)

_____
_____
_____
_____
_____
_____
_____
_____

Body Cavities (fat stores, pleura, thymus, lymph nodes)

Spleen

Respiratory System (trunk passages, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes; submit lung lesions for TB culture; **bronchial lymph nodes should be cultured for TB even if normal in appearance)**

Cardiovascular System (heart, pericardial sac, great vessels, myocardium, valves, chambers)

Digestive System (mouth, teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, rectum, liver, pancreas, mesenteric lymph nodes)

Urinary System (kidneys, ureters, bladder, urethra)

Reproductive System (testes/ovaries, uterus & cervix, penis/vagina, urogenital canal, prostate, seminal vesicles, bulbo-urethral gland, mammary gland, placenta)

Endocrine System (thyroids, parathyroids, adrenals, pituitary)

Central Nervous System (brain, meninges, spinal cord)

Sensory Organs (eyes, ears)

Additional Comments or Observations:

Prosector:_____Date:_____

Summarize Preliminary Diagnoses:

_____

_____

_____

Laboratory Studies: Please attach results of cytology, fluid analysis, urinalysis, serum chemistries, bacteriology, mycology, virology, parasitology, x-ray, photographs, or other data collected.

TISSUE CHECK LIST

Freeze 3-5 cm blocks of tissue from lesions and major organs (e.g., lung, liver, kidney, spleen) in small plastic bags. Freezing at -70 degrees Celsius in an ultra-low freezer is preferred. If this is unavailable, freezing at conventional temperatures is acceptable (use a freezer without an automatic defrost cycle if possible).

Any lesions noted in the lungs should be submitted to NVSL or other qualified mycobacterial laboratory for mycobacterial culture. Bronchial lymph nodes should be cultured for TB even if normal in appearance. Preserve as many of the tissues listed below as possible in 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts solution. Tissues should be no thicker than 0.5 to 1.0 cm. Fix diced (1x1 mm) pieces of kidney, liver, spleen and lung in a suitable EM fixative if possible - glutaraldehyde base e.g., Trump-McDowell fixative. NOTE: There is generally no need to fix and label each tissue separately. Take 2 sets of fixed tissue. Bank one set. Send tissues required for diagnosis to primary pathologist and request a duplicate set of slides for the SSP pathologist, Dr. Richard J. Montali who should be contacted for further instructions. Also, freeze post mortem serum (from heart), urine and any abnormal fluid accumulations. Consult **Elephant Research and Tissue Request Protocol** for specific project sample requests.

Adrenal	Kidney	Penis	Thymus
Blood *	Large intestine	Pituitary	Tongue
Bone with marrow	Liver	Prostate	Trachea
Bulbo-urethral gland	Lung	Salivary gland	Trunk cross section
Brain	Lymph nodes (external & internal)		Seminal vesicles
			Uterus/cervix
Cecum	Mammary gland	Skin	Ureter
Diaphragm	Muscle	Small intestine	Urinary bladder
Esophagus	Nerve (sciatic)	Spinal cord	Vaginal/urogenital canal
Eye	Ovary/testis	Spleen	
Heart/aorta	Pancreas	Stomach	
Hemal node	Parathyroid	Temporal gland	

***** Collect post mortem blood, separate serum and freeze for retrospective studies.

Primary Pathologist (Name): _____

Lab _____

Address _____

_____

Phone _____

(Please send a copy of this protocol with gross descriptions and preliminary diagnoses to SSP pathologist. Send final report with histopatholgic findings and any pertinent digital or color slides to):

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INDIVIDUALS INTERESTED IN PARTICIPATING IN NECROPSY PROCEDURES

The following people may be available to participate in necropsies. If you are interested, please contact them as soon as possible after an animal dies or before euthanasia.

Name	Work Number	Home Number	Fax Number
Dr. R.J. Montali Washington, DC Email: montalir@nzp.si.edu	(202) 673-4869 Cell: (202) 863-0186	703-718-2870	(202) 673-4660
Genevieve Dumonceaux, DVM Busch Gardens, Tampa, Florida Email: genevieve.dumonceaux@buschgardens.com	(813) 987-5561	(831) 907-5795	(813) 987-5548
L. E. L. (Bets) Rasmussen Beaverton, Oregon Email: betsr@bmb.ogi.edu	(503) 748-1263 Cell: (503) 705-3719	(503) 621-1435	(503) 748-1464
Susanne J. Miller Pocatello, Idaho Email: ski@inel.gov	(208) 526-0938	(208) 529-2453	(208) 236-4600
D. J. Hillman Baton Rouge, Louisiana Email: dhillmann@mail.vetmed.lsu.edu	(504) 346-3246	(504) 272-0156	(504) 346-3329
Scott Terrell, DVM, DACVP Orlando, Florida Email: Scott.P.Terrell@disney.com	(407) 938-2746	(407) 238-0693	(407) 938-1909
Vaughan A. Langman Shreveport, Louisiana Email: 75554,3044@compuserv.com	(318) 797-5244	(318) 798-2750	(318) 797-5222